IN THE CLAIMS

Please replace claim 4 with the following:

a9

4. The combination of claim 2, wherein the plant is an ornamental plant.

REMARKS

Any fees that may be due in connection with this application throughout its pendency may be charged to Deposit Account No. 50-1213.

Claim 4 is amended to restore proper claim dependency. No new matter has been added.

The specification is amended to correct obvious typographical, spelling and formatting errors. No new matter has been added.

In view of the amendments and above remarks, entry of the amendments and examination of the application on the merits are respectfully requested.

* * *

Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: BRYAN

Serial No.: 09/803,211

Filed: March 8, 2001

For: *BIOLUMINESCENT NOVELTY ITEMS*

Art Unit: Unassigned

Examiner: Unassigned

I hereby certify that this paper and the attached papers are being deposited with the United States Postal Service as first class mail in an envelope addressed to:
Commissioner for Patents
Washington, D.C. 20231, on this date.

05/16/01
Date

Kelly M. Fischer

ATTACHMENT TO THE PRELIMINARY AMENDMENT MARKED UP PARAGRAPHS AND CLAIMS (37 CFR §1.121)

IN THE SPECIFICATION:

Please amend the specification as follows:

Please amend the paragraph on page 13, line 32, to page 14, line 4, as follows:

2. Capsules, pellets, liposomes, micronized particles
   a. Encapsulating vehicles-in general
   b. Encapsulating vehicles -liposomes
   c. Encapsulating vehicles -gelatin and polymeric vehicles
   d. Endosomes and vacuoles
   [d]e. Micronized particles

Please amend the paragraph on page 52, lines 16-28 as follows:

Bacterial luciferase, as exemplified by luciferase derived from *Vibrio harveyi* (EC 1.14.14.3, alkanol reduced-FMN-oxygen oxidoreductase 1-hydroxylating, luminescing), is a mixed function oxidase, formed by the association of two different protein subunits α and β. The α-subunit has an apparent molecular weight of approximately 42,000 kD and the β-subunit has an apparent molecular weight of approximately 37,000 kD (see, *e.g.*, Cohn *et al.* (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*:[102]120-123). These subunits associate to form a

2-chain complex luciferase enzyme, which catalyzes the light emitting reaction of bioluminescent bacteria, such as *Vibrio harveyi* (U.S. Patent No. 4,581,335; Belas *et al.* (1982) *Science 218*:791-793), *Vibrio fischeri* (Engebrecht *et al.* (1983) *Cell 32*:773-781; Engebrecht *et al.* (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*:4154-4158) and other marine bacteria.

Please amend the paragraph on page 61, lines 8-24 as follows:

Two classes of phycobiliproteins are known based on their color: phycoerythrins (red) and phycocyanins (blue), which have reported [absorbtion] absorption maxima between 490 and 570 nm and between 610 and 665 nm, respectively. Phycoerythrins and phycocyanins are heterogenous complexes composed of different ratios of alpha and beta monomers to which one or more class of linear tetrapyrrole chromophores are covalently bound. Particular phycobiliproteins may also contain a third γ-subunit which often associated with $(\alpha\beta)_6$ aggregate proteins.

All phycobiliproteins contain either phycothrombilin or phycoerythobilin chromophores, and may also contain other bilins, such as phycourobilin, cryptoviolin or a 697 nm bilin. The γ-subunit is covalently bound with phycourobilin, which results in the 495-500 nm absorbance peak of B- and R-phycoerythrins. Thus, the spectral characteristics of [phycobiliproetins] phycobiliproteins may be influenced by the combination of the different chromophores, the subunit composition of the apo-phycobiliproteins and/or the local environment that affects the tertiary and quaternary structure of the phycobiliproteins.

Please amend the paragraph on page 62, lines 3-5 as follows:

As noted above, these proteins may be used in combination with other [fluoresent] fluorescent proteins and/or bioluminescence generating systems to produce an array of colors or to provide different colors over time.

Please amend the paragraph on page 77, lines as follows:

Synthetic matrices include, but are not limited to: acrylamides, dextran-derivatives and dextran co-polymers, agarose-polyacrylamide blends, other

polymers and co-polymers with various functional groups, methacrylate derivatives and co-polymers, polystyrene and polystyrene copolymers [see, *e.g.*, Merrifield (1964) Biochemistry *3*:1385-1390; Berg *et al.* (1990) in *Innovation Perspect. Solid Phase Synth. Collect. Pap.*, Int. Symp., 1st, Epton, Roger (Ed), pp. 453-459; Berg *et al.* (1989) in *Pept., Proc. Eur. Pept. Symp.*, 20th, Jung, G. *et al.* (Eds), pp. 196-198; Berg *et al.* (1989) *J. Am. Chem. Soc. 111*:8024-8026; [Mitchell] Kent *et al.* (1979) *Isr. J. Chem. 17*:243-247; [Kent] Mitchell *et al.* (1978) *J. Org. Chem. 43*:2845-2852; Mitchell *et al.* (1976) *Tetrahedron Lett. 42*:3795-3798; U.S. Patent No. 4,507,230; U.S. Patent No. 4,006,117; and U.S. Patent No. 5,389,449]. Methods for preparation of such matrices are well-known to those of skill in this art.

Please amend the paragraph on page 116, lines 25-29 as follows:

Alternatively, the board and pieces may include adsorbed or absorded [lyophillized] lyophilized bioluminescence-generating reagents. Contacting these items with water, containing the appropriate salts and buffers, such as calcium, if for example, the aqueorin system is used, or ATP if the firefly system is used.

Please amend the paragraph on page 131, lines 8-26 as follows:

The resulting fish are fed food containing an appropriate luciferin or luciferins [or luciferase] and any additional bioluminescence generating reagents required. Typically, the luciferin will be present in the fish food at concentrations ranging from about 1 part per million (ppm) to about 1 part per 10, weight/weight. As the luciferin, bioluminescent activators and other system components come in contact with the luciferase expressed by the transgenic fish, the fish or selected organs or tissues will glow. For example, if the luciferase is expressed on the tissues lining the transgenic fish's mouth, then its mouth will light up as it eats the fish food. Similarly, if the fish transfected with the luciferase gene is [transluscent] translucent, then the digestive organs, particularly the stomach, will glow as the bioluminescence generating components come into contact and complete the bioluminescent reaction. The

selected luciferase/luciferin systems should be one that is resistant to conditions, such as the acidic pH of the digestive system, in the fish.

Thus, for purposes herein, fish food that includes luciferin, preferably in [lyophillized] lyophilized form, particularly, *Renilla* coelenterazine and *Vargula* luciferin, is provided. The transgenic fish that express luciferase or luciferin are also provided.

Please amend the paragraph on page 160, line 23 to page 162, line 13 as follows:

The matrix material 1034 may be any porous material to which the bioluminescence generating component can be adsorbed, absorbed or otherwise linked, as described herein, that is non-reactive with the components of the bioluminescence generating system. When necessary, the matrix material 1034 is included and bathed in the fluid 1030 such that the component(s) of the [bioluminescnce] bioluminescence generating system affixed to the matrix material are released into the fluid 1030. As the piston is continually advanced, the fluid, containing bioluminescence generating components eluted from the matrix material, is forced through the filter 1036 and out the nozzle 1038 and aperture 1040. Filter 1036 is used to prevent the expulsion of matrix material 1034 from the second cylinder 1014. As a result, the filter 1036 may be made from a cloth or metallic weave, or any other material that will not react with the various components and compositions present within the second cylinder 1014.

It is to be appreciated, however, that the various components of the bioluminescent reaction may be distributed in different combinations between the two cylinders 1010, 1012, and the matrix material 1034. One cylinder, such as the first cylinder 1010, typically contains the dry or condensed ingredients 1018 and the second cylinder 1012 typically contains a fluid 1030 and the matrix material containing the remaining components necessary for the bioluminescent reaction. The dry or condensed ingredients may contain any combination of the components of the bioluminescence generating system, such as a luciferase and/or a luciferin, buffer salts, ATP, Ca^{2+} or any other necessary

activator. The fluid 1030 may be water, a buffer, an organic solvent or any other aqueous medium suitable for solubilizing or suspending one or more components of a bioluminescence generating system to be dispensed into the bioluminescent novelty item.

In a preferred embodiment, the dry ingredients 1018 include [lyophillized] lyophilized luciferase and buffer salts in powder form, and the fluid includes an alcohol that is used to dissolve or suspend a quantity of luciferin affixed to the matrix material. Alternatively, all of the components of a bioluminescence generating system, such as the *Vargula* system, may be added and packaged in the first and/or second cylinders in the absence of molecular oxygen such that components are activated when combined and exposed to air.

Referring now to FIGURE 30, the cartridge 1000 is shown as used in conjunction with a typical bioluminescent novelty item 1042. As shown, the plunger 1004 has been pressed completely against the block 1002 causing the first piston 1006 and the second piston 1008 to be inserted completely into the block 1002. As the piston 1006 is advanced into the block 1002, the dry or condensed ingredients 1018, for example, are forced out of the first cylinder 1010, through the funnel 1020 thereby breaking the seal 1022, and out the nozzle 1024 and aperture 1026 into the chamber 1044 in novelty item 1042. Likewise, as the piston 1008 is advanced into the block 1002, the seal 1032 on the sleeve 1014 is ruptured causing the fluid 1030 to be dispensed, optionally bathing matrix material 1034. As the piston 1008 is advanced further, the fluid 1030 is forced through filter 1036, out nozzle 1038 and aperture 1040, and into chamber 1046 of novelty item 1042. In this manner, the novelty item is fully recharged with the components of a [bioluminescnce] bioluminescence generating system necessary for a bioluminescent reaction, while maintaining the separation of the chemicals as required for some novelty items.

IN THE CLAIMS

Please amend claim 4 as follows:

4. The combination of claim [1] 2, wherein the plant is an ornamental plant.

* * *

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